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PRODUCTION METHOD OF DITERPEN ALCOHOL

[ジテルペンアルコールの製法]

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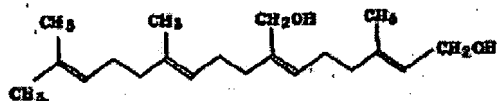
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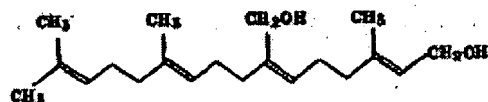
1. [Title of the Invention] Production method of diterpen alcohol

2. [Claims] The production method of diterpen alcohol characterized such that the plant genus Croton is extracted by solvent and using extract solution, the compound with the following chemical formula is extracted:



3. [Detailed explanation of the invention]

The present invention relates to the novel production method of diterpen alcohol, in more detail, to the method of separating and collecting the digestive ulcer resistant action substance expressed by the following chemical structure (I).



The plant genus *Croton* is a plant that belongs to Euphorbiaceae, and many plants in the genus have been used as a herbal drug since old times, however, one whose structural research has been done in detail is only the seed (herbal drug name: hamame) of *Croton Tiglium* L. Particularly, the research of physiological active ingredient regarding the root and material of the plant genus *Croton* was hardly done. The inventors herein have been engaged in the R&D of novel drugs by isolating the pharmacologically active plant ingredient

for many years, a novel diterpen alcohol was isolated that is contained in the plant genus Croton, particularly, Plau-noi (Croton Colummaris Airy Shane, alias, Croton joufra Roxb.), Plau-yai (Croton oblogifolium Roxb.) and Plau-yai (Croton oblongifolius Roxb.) and Plau-iat (Croton HUTchinsonianus Hosseus) that are herbal drugs produced in Thailand, and succeeded in determining its chemical structure, and found out that this compound is the effective ingredient that shows digestive ulcer resistant action, thus completing the present invention. The present invention is characterized such that the plant genus Croton was extracted by solvent,

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The compound that has the formula (I) is extracted from the extract solution. Regarding the method of the present invention, as the solvent used for extraction, the organic solvent used for extracting water or regular plant ingredients such as the following ,for instance, methanol, ethanol, isopropanole and the like that are alcohol type, ethyl ether, isopropyl ether and the like that are ether type, dichlor methane, dichlor ethane, chloroform and the like that are halogenated hydrocarbon type, methyl acetate, ethyl acetate and the like that are ester acetate, acetone, methyl ethyl ketone and the like that are lower alkyl ketone and the like, benzene, toluene and the like that are aromatic hydrocarbon type and the like can be used without limitation. In order to obtain the fractionation

that contains aforementioned effective ingredient by this extract solution, the method of collecting a regular plant neutral ingredient is used. Furthermore, in order to isolate the compound with aforementioned chemical structure (I), the regular neutral ingredient separation method is used; however, it is advantageous to isolate by combining column chromatography or by crystallizing as a derivative. That is, for instance, in order to remove the lipid from aforementioned extracted product, extraction liquid or condensed product is washed by hydrocarbon such as n-hexane, furthermore, is dissolved in benzene or ether that are water non mixing solvent, then, washed by sodium bicarbonate water that are bicarbonate alkaline water solution, sodium carbonate water solution, potassium carbonate water solution and the like that are carbonate alkaline water solution or sodium hydroxide water solution, potassium hydroxide water solution that are hydroxide alkaline water layer thus, acid ingredient is removed, organic solvent layer is dried by sodium sulfate anhydride and when solvent is distilled and removed from the solvent, organic solvent dissolvable neutral substance fractionation that has digestive ulcer resistance action can be obtained. Furthermore, in order to isolate the targeted effective ingredient, the filler such as silica gel, alumina, silic acid and the like are used, as an expanding agent, one type or two types or more from benzene, ethyl ether, ethyl acetate, chloroform, acetone, alcohol and the like that are organic solvent are mixed,

making mixing solvent, or the mixing solvent of above described solvent and petroleum type solvent are used for column chromatography. When the solvent is distilled and removed from the eluent solution, the target compound is obtained, however, if necessary, it can be refined more by a regular method or forming derivative or distillation by pressure reduction. That is, the neutral ingredient obtained by plant extract is treated with crystalline derivative synthesis reagent used for alcohol compound normally, for instance, 3, 5- dinitro benzoyl chloride, phthalate anhydride, phenyl isocyanate and the like, thus is isolated as crystalline derivative, this is hydrolyzed, thus isolating the target compound. The physico chemical characteristics of the targeted diterpen alcohol represented by the aforementioned chemical structure (I) obtained by the method of the present invention is as follows. Substance name: (E. Z. E) -7- hydroxy methyl -5, 11, 15 trimethyl - 2, 6, 10, 14 - hexa decatetraen -1- ole; Molecular formula: $C_{20}H_{34}O_2$; Infrared ray absorption spectrum: γcm^{-1} (solution film): 3300, 1665, 1440, 1380, 1000. Nuclear magnetic resonance spectrum: δppm (CCl_4): 158 (6H, singlet), 166 (6H, singlet) 1.9-2.5 (12H, multiplet), 3.94 (2H, singlet), 3.97 (2H, doublet), 5.0-5.3 (4H, multiplet). The target compound expressed by the aforementioned structural formula (I) obtained by the method of the present invention and the neutral fractionation that can dissolve the organic solvent

that contains this show superior digestive ulcer resistance action in ether case. The following will explain its pharmacological effect.

1...The anti-reserpine ulcer action in mice this was done according to the method of Blackmann 1). The sample was administered into the stomach cavity of the ddy type male mouse (weight 28~33g), and after 30 minutes, reserpine 10mg/kg was administered subcutaneously. 18 hours after administering, the animals were killed, the stomach was extracted, and the stomach was expanded by 0.5% formalin 2ml, and was fixed. After fixing, the stomach was cut opened along the [illeg], and the ulcer area was measured under the stereo graphic microscope. The ulcer area of the sample - administered group and that of the control group were compared, the inhibition rate was computed. /3

1) .J. C.Blackmann, D.S. campion, F.N. Fastier, Brit. J. Pharmacol.. 14, 112 (1959).

Drug	Used amount mg/Kg i.p	Animal number N	Inhibitory rate%
(I)	100	5	68*
(II)	100	5	57*
Gefarnate	100	5	10

*P< 0.05

2). H. Selye and S. Szabo. Nature 244, 458 (1973)

Drug	Used amount mg/Kg/day x 4	Animal number N	Ulcer coefficient	Inhibitory rate%
Contrast	-	20	2.45	-
(I)	100	10	2.00	18
(II)	300	18	1.56	34*
Gefarnate	300	10	2.20	10

* <0.05

2. Anti-cyteramine duodenal ulcer This was done according to the method of Selye and Szabo 2). Female Donryu rats (200~ 220g) fasted overnight and cysteamine 300mg/kg was administered orally, duodenal ulcer was caused. The sample was orally administered 2 days before administering cysteamine and right before administering and one day after administering it. The animals were killed 2 days after administering cysteamine, and duodenal ulcer coefficient was found.

The ulcer coefficient of the sample administer group and that of the control group were compared, thus seeking the inhibition rate.

Next, using embodied examples, the method of the present invention will be specifically explained, however, the method of the present invention is not limited to these.

(Embodied example 1) Pulverized herbal drug Plau-noi 28 kg produced in Thailand is heated and extracted under the circulating current three times using methanol. The extract solution is collected and methanol is distilled and removed, extract 672g is obtained. Extract is dissolved in 90% methanol 5 liter, and washed by n- hexane, and then methanol is distilled and removed. The remaining solution is suspended in 3 liter of water, and extracted by ethyl ether; extracted solution is washed by 5% sodium carbonate, then, is dried by sodium sulfate anhydride. When the solvent is distilled and removed from the solution, 117g of oily product remains. Above described oily substance is absorbed into the column that used 1.5mg of silica gel, and it is initially eluted by 10% ethyl acetate containing benzene, then, it is eluted by 30% ethyl acetate containing benzene. If the part that contains the effective ingredient in the eluent fractionation of 30% ethyl acetate containing benzene is distilled and removed, targeted diterpen alcohol 17g is obtained as the colorless consistent oil.

(Embodied example 2) Pulverized herbal drug Plau-noi 1kg produced in Thailand is heated and extracted under the circulating current three times using acetone. The extract solution is collected and acetone is distilled and removed, extract 19g is obtained. Extract is dissolved in 90% methanol 100ml, and washed by n- hexane, and then methanol is distilled and removed. The remaining solution is suspended in 100ml of water, and extracted by ethyl ether; extracted

solution is washed by 5% sodium carbonate water, then, is dried by sodium sulfate anhydride. When the solvent is distilled and removed, 12g of residual oily substance is dissolved in pyridine 10ml and by adding 25g of 3, 5-dinitro benzoyl chloride, it is left alone for 5 hours at the room temperature. Water is poured into the reaction mixture, and extraction is done by ethyl ether. ether [illeg] is washed by 5% sodium bicarbonate water, next, washed by diluted hydrochloric acid, then, is dried by sodium sulfate anhydride. Methanol is added to the condensed ether, and left alone. Precipitated sediment is collected, and if recrystallization is done from ethyl ether and methanol mixed solution, the targeted bis- 3, 5 -dinitro benzoate (melting point 85-4 deg C) 1g is obtained. Analysis value (%) C: 58.69, H: 5.59, N: 2.97, as C₃₄ H₃₈ O₁₂ N₄, the computation values (%) are C; 58.84, H: 8.52, N: 8.06. Bis-3,5.-dinitro benzoate thus obtained is dissolved in dioxane 5ml and it is hydrolyzed by 5% sodium hydroxide, and is extracted by ethyl ether. Extract solution is washed by 5% sodium carbonate, then, is dried by sodium sulfate anhydride. When solvent is distilled and removed, targeted diterpen alcohol 500mg is obtained./4

(Embodied example 5) Pulverized herbal drug Plau-noi 1kg produced in Thailand, while being heated and churned, is extracted three times. Extraction solution is collected, water is distilled and removed as much as possible under the pressure reduction, methanol 250 ml

is added to the residual extract, churned and filtered. Filtered water is washed by n-hexane, methanol is distilled and removed. the solution is suspended in 150ml of water, is extracted by isopropyl ether, and extract solution is washed by 5% sodium carbonate water, then, is dried and solvent is distilled and removed. The residual organic solvent neutral fractionation 18g is refined by the column chromatography according to the method of embodied example 1, the targeted diterpen alcohol 580 mg is obtained.